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Isolation and characterization of microsatellites in yellow perch (*Perca flavescens*)

L. LI*, † H. P. WANG, † C. GIVENS*, S. CZESNYŚ and B. BROWN*

*Ecological Genetics Laboratory, Virginia Commonwealth University, Richmond, Virginia 23284-2012, USA, †Aquaculture Genetics and Breeding Laboratory, Ohio State University, South Centers, 1864 Shyville Road, Piketon, Ohio 45661, USA, ‡Experimental Marine Biology Laboratory, Institute of Oceanology Chinese Academy of Science, 7 Nanhai Road, Qingdao, 266071, China, §Illinois Natural History Survey, Lake Michigan Biological Station, 400 17th Street, Zion, IL 60099, USA

Abstract

A total of 45 microsatellite loci from yellow perch, *Perca flavescens*, were isolated and characterized. Among the 45 microsatellite loci, 32 had more than two alleles. A wild population of *P. flavescens* ($n = 48$) was used to examine the allele range of the microsatellite loci. Mendelian inheritance of alleles was confirmed by examining the amplified products in pair-mated families. The number of alleles for the 32 polymorphic loci varied from two to 16, and observed heterozygosity ranged between 0.024 (YP79) and 0.979 (YP60). Cross-species polymorphic amplification in four other Percidae species was successful for 22 loci.

Keywords: microsatellite, *Perca flavescens*, tetranucleotide, trinucleotide, yellow perch

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Markers

The yellow perch, *Perca flavescens*, is an ecologically and economically important freshwater fish native to the Nearctic zone (Nelson 1976), widely introduced, and frequently cultivated for supplementation and commodity production. Genetic management of the species is underway to address dramatic reductions in native population sizes, to track the spread of introduced populations, and to aid efforts in marker-assisted selection and broodstock improvement. Previously, 10 dinucleotide repeat microsatellite loci were reported for *P. flavescens* (Leclerc *et al.* 2000) and it was suggested that the levels of variation exhibited qualified them as useful tools for selective breeding programs and evaluating fine-scale population structure. Microsatellite markers have demonstrated utility in yellow perch for detecting population processes (Miller 2003). Due to the hypervariable nature of dinucleotide repeats in general, combined with difficulties in amplifying and scoring previously published dinucleotides for this particular species, we focused the majority of our efforts on developing a suite of tri- and tetranucleotide repeats for yellow perch.

Repeat-enriched microsatellite libraries were constructed following the protocol of Karp *et al.* (1998) with some modification. The genomic DNA from fin tissues of *P. flavescens* was *Sau3A* digested and fragments of 0.5–2 kb were selected and ligated to adaptor SAULA (5'-GCGGTAC-CCGGGAAGCTTGG-3') and SAULB (5'-GATCCCAAGCTTCCCGGTACCGC-3'). Enrichment was performed using two cocktails of biotinylated repeat motifs [(CA)_n and (GT)_n] and [(AAC)₁₁ (GAAT)₁₀ (ACAT)₁₁ (AAAG)₁₁ (GTA)₁₅ (AAT)₁₅] and then selected from the mixture using Vectrex Avidin D. The resulting fragment population enriched for microsatellites was ligated to TOPO vector (Invitrogen). Of approximately 100 repeat sequences examined, 72 were found to have sufficiently high-quality flanking regions (more than 50 bp) and moderate numbers of tandem repeats to be selected for primer design. The shorter of each primer pair was modified at the 5' end to include a universal tail (5'-CAGTCGGGCGTCATCA-3') as described by Boutin-Ganach *et al.* (2001). Amplification of microsatellite loci was performed with three primers, the tailed primer, the nontailed primer, and a universal 5'-labelled (FAM, TET or HEX) primer having the same sequence as the universal tail. Polymerase chain reaction of 6 µL contained 3 µL of JumpStart RedMix (Sigma), 1.5 pmol of both nontailed and labelled primers, and 0.1 pmol of the tailed primer, 25 ng DNA, in the presence

Correspondence: B.L. Brown, Fax: +804-828-0503;
E-mail: blbrown@vcu.edu

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of 100 μ M spermidine. Amplification was conducted in PTC-100 thermal cyclers (MJ Research) using an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s denaturation at 94°, 30 s annealing at a locus-specific temperature (Table 1), 30 s extension at 72°, and a final 5-min extension at 72°. Amplification products were resolved on ultrathin polyacrylamide using a BaseStation 51 DNA fragment analyzer (MJ Research). Mendelian inheritance of alleles was determined by examining the amplified products in several full-sib families per locus (both parents and 14 offspring). To determine allele range and population-level variability, a wild group of *P. flavescens* from Saginaw Bay Michigan was examined ($n = 48$). Primers also were tested with the following related species: sauger, *Sander canadense* ($n = 5$); walleye, *Sander vitreus* ($n = 3$); Eurasian perch, *Perca fluviatilis* ($n = 5$); and Shield darter, *Percina peltata* ($n = 2$). Statistical analyses were performed using GENEPOP version 3.4 (Raymond & Rousset 1995) using default settings for dememorization, batches, and iteration.

Of 72 primer sets tested, 45 produced reproducible products within the expected size range. Of these, 13 loci yielded homologous products (YP1, YP10, YP12, YP15, YP26, YP31, YP33, YP52, YP59, YP69, YP92, YP93, YP98) and 32 loci were polymorphic (Table 1). The number of alleles for each locus ranged between two and 16; the dinucleotide repeats YP68 and YP106 had the highest numbers of alleles, 15 and 16, respectively. Observed heterozygosity (H_O) of the 32 polymorphic loci ranged from 0.024 (YP79) to 0.979 (YP60). Twenty of the 32 polymorphic loci exhibited Mendelian segregation; however, the remaining 12 loci that appear distorted may have resulted from the limited number of progeny tested from the reference families. Considering allele frequencies and linkage estimates, the possibility of null alleles at locus YP110 was strongly indicated. Of 464 comparisons, no other significant evidence for linkage was observed among these loci except for the pair YP 60 and YP 96 ($P = 0$). Ultimately, only six of the 32 loci (YP6, YP13, YP16, YP17, YP71, and YP110) conformed to the expectations of Hardy–Weinberg equilibrium at $P > 0.05$.

Table 1 Repeat structure, primer sequences (universal tails italicized), amplification characteristics (T_a , annealing temperature), and polymorphism data for microsatellite loci developed for *Perca flavescens*. Observed numbers of alleles in a wild population, heterozygosity values (observed and expected), and the P values for exact tests of fit to Hardy–Weinberg equilibrium (HWE) are also shown

Locus repeat	GenBank Accession no.	Primer sequences (5'–3')	T_a (°C)	Cloned allele size (bp)	Observed no. of alleles	H_O	H_E	HWE
YP6 (AGT) ₁₀	DQ826678	<i>CAGTCGGGCGTCATCAAAGTAGCAGATG</i> <i>TAAAAGAGCAAGAAA</i> <i>GGGCAAGAGACAGAAAGCCAATA</i>	57	214	2	0.087	0.084	1.000
YP7 (AAC) ₇ AAT (AAC) ₂	DQ826679	<i>ATGTATTTCTGTCAACTGGCGG</i> <i>CAGTCGGGCGTCATCAGAATGTGT</i> <i>CCTTATTTGCGTGG</i>	55	183	3	0.771	0.487	0.000
YP9 (ACT) ₉ AAT(ACT) ₂	DQ826680	<i>CAGTCGGGCGTCATCATTTGAGCAGACAGG</i> <i>GCAGAGA</i> <i>CCCCTTTCAACTCCACCACT</i>	58	157	3	0.958	0.514	0.000
YP13 (GTA) ₁₁	DQ826683	<i>GGCACCCAAACTACCACT</i> <i>CAGTCGGGCGTCATCAAACAAGCCCCATACA</i>	55	233	4	0.468	0.455	1.000
YP16 (CATT) ₉	DQ826685	<i>CAGTCGGGCGTCATCAGTGTGTGGGTTA</i> <i>CTGCTGGC</i> <i>TCCCTCTCTCTCCCTTTCA</i>	57	303	3	0.333	0.389	0.360
YP17 (TAG) ₁₀ TANGTG(TAG) ₂	DQ826686	<i>CAGTCGGGCGTCATCACAGCGTTTCCACAGTATTGACC</i> <i>GGGTTTTACACTGTTGATGGGAT</i>	55	209	5	0.630	0.614	0.096
YP28 (AGT) ₈ AATAT (AGT) ₂	DQ826688	<i>TGCTACAACCTTTCGCCTCAA</i> <i>CAGTCGGGCGTCATCAAACAA</i> <i>CAACTGCAACTACCA</i>	55	196	2	0.700	0.810	0.040
YP30 (TTCT) ₆	DQ826689	<i>CAGTCGGGCGTCATCAACATCTATCTC</i> <i>ACTTTCATTTACATTACATCTTCTCTC</i> <i>TTCTCAAATCCT</i>	55	97	5	0.896	0.561	0.000
YP41 (TCTT) ₁₁	DQ826692	<i>CGTCCCTCCCTCTATCC</i> <i>CAGTCGGGCGTCATCATTG</i> <i>CTGTGCTGCCATTTTC</i>	55	175	6	0.375	0.708	0.000
YP49 (TAG) ₁₂	DQ826693	<i>ATCAGACTGACGACGGCA</i> <i>CAGTCGGGCGTCATCACTCGG</i> <i>ACAATGGCAAACCT</i>	57	124	7	0.833	0.812	0.000

Table 1 Continued

Locus repeat	GenBank Accession no.	Primer sequences (5'-3')	T_a (°C)	Cloned allele size (bp)	Observed no. of alleles	H_O	H_E	HWE
YP55 (TCTT) ₁₀	DQ826695	CCCTCCTCTTGTGTGTGTGC CAGTCGGGCGTCATCAGCTCTGAGT CTGCCTTTGTT	55	256	5	0.521	0.794	0.000
YP60 (AGAA) ₁₀	DQ826697	ATGTGTTATATGCTTTGCGTA CAGTCGGGCGTCATCAGCTG TTCTGTAAATGTGTTG	50	195	12	0.979	0.902	0.000
YP62 (CATA) ₁₀	DQ826698	CAGTCGGGCGTCATCATTGAGGTGAGGTA TTGGTTTATGAGAAGGGAGGTGTGTGC	57	215	6	0.813	0.740	0.000
YP65 (AAAG) ₁₂	DQ826699	CAGTCGGGCGTCATCAGAAGGAATGAAA GAATGAGGTCCCTCCATCTCTGTCTGTG	55	284	5	0.917	0.766	0.000
YP66 (TTCT) ₁₁	DQ826700	CAGTCGGGCGTCATCACTGCTGATGAA GTGGACAACATAGGGGTCAGGGCAAAC	55	283	3	0.095	0.156	0.015
YP68 (AC) ₅ GCAC GC (AC) ₅ AT(AC) ₉	DQ826701	GACAGAAAGCAAGAAGGGAA CAGTCGGGCGTCATCAATCCTTTTTC TCCAATCCTGA	55	210	16	0.542	0.778	0.000
YP71 (GTT) ₁₀ GTA CTT	DQ826703	CAGTCGGGCGTCATCATTTGTGCCG ATGAGCAGTTAAAACCACACCGAACATCCAA	55	231	4	0.688	0.654	0.405
YP73 (CAA) ₁₆	DQ826704	CAGTCGGGCGTCATCAGATGGGAGG AAATGGTGAGAGAACGCCAAGCCTGAAT	55	151	4	0.103	0.460	0.000
YP78 (GTA) ₁₃	DQ826705	GCAGCCCCTACAATGGTT CAGTCGGGCGTCATCAGCCTT CTTCTGTTATTTTTC	55	196	8	0.625	0.764	0.000
YP79 (AC) ₅ AA(AC) ₉	DQ826706	CTCCAACAGTCAACAGGTAACA CAGTCGGGCGTCATCATCCA TTCTTTACTGCTTTCTA	55	149	3	0.024	0.402	0.000
YP80 (TAC) ₂ TAA(TAC) ₁₆	DQ826707	TGTCAGAGTAAAGATGAGCCCA CAGTCGGGCGTCATCATGGACCTTT GGGATTACTTTTA	57	209	3	0.667	0.597	0.000
YP81 (ACA) ₉	DQ826708	CAGTCGGGCGTCATCACACGAAGGGAATC AAGTTTTTCATTTACAAACATTTCTGCCAT	57	269	10	0.958	0.882	0.000
YP84 (CAA) ₈ (CAG) ₃ (CAA) ₃ CAG	DQ826709	AAATTGATGCACCACCCTT CAGTCGGGCGTCATCAAGG AGGGAGGTCTCTGCTTT	57	189	3	0.708	0.547	0.000
YP85 (TCTT) ₁₅	DQ826710	CCCAGCCCTTCGTTTTA CAGTCGGGCGTCATCAGC CCCTTACAAAGCATCA	55	187	5	1.000	0.794	0.000
YP96 (ACA) ₁₃	DQ826713	CTAACACAAGTTTCCACCCG CAGTCGGGCGTCATCAGAACC ATAAATCACCTTCTAAT	55	144	7	0.896	0.757	0.000
YP99 (GATA) ₁₅	DQ826715	ACACAGAGCAATACCATCGTCA CAGTCGGGCGTCATCAAGCAAC TGTAATGTTCCCTCCAAA	55	279	5	0.521	0.763	0.000
YP106 (GT) ₁₆ (GA) ₃ (GGA) ₆ (GT) ₄	DQ826716	CAGTCGGGCGTCATCACAAGGAGACTTTA CCCCAGGTTTTCCGATGTGGTAAGGCA	55	357	15	0.708	0.759	0.000
YP108 (TAC) ₁₂	DQ826717	TGTCATAGCAGAACAACACCTT CAGTCGGGCGTCATCATTCTT CATTACCCTGGTTTCT	55	173	4	0.375	0.718	0.000
YP109 (ACA) ₁₆	DQ826718	CAGTCGGGCGTCATCATTCCAGAGTT GGCAAGACTCATGTTCCTGTTGCTTCA	55	147	6	0.896	0.828	0.000
YP110 (TTG) ₁₈	DQ826719	CAGTCGGGCGTCATCATTGAGCCCTTC ACTTTTGTATCAGAGCAATGACCAAGCC	55	207	4	0.646	0.687	0.121
YP111 (CTA) ₁₆ (ATA) ₁₈	DQ826720	CAGTCGGGCGTCATCATGTGTATGGCTATT GTGCTCTTTGTTTCAGTGTPTTTTCGC	53	251	5	0.813	0.763	0.000
YP113 (GT) ₁₇	DQ826721	CAGTCGGGCGTCATCAGGTTGGGACAC AGAGACACTGGTGTGGATTGGGGCAT	57	130	11	0.854	0.891	0.000

Table 2 Cross-amplification data for four other Percidae: *Sander canadense*, *Sander vitreus*, *Perca fluviatilis* and *Percina peltata*. Numbers in parentheses refer to observed allele size range in bp. 'M' indicates amplification produced monomorphic allele; '-' indicates no amplification observed

Loci	<i>S. canadense</i> n = 5	<i>S. vitreus</i> n = 3	<i>P. fluviatilis</i> n = 5	<i>P. peltata</i> n = 2
YP6	M	M	M	—
YP7	—	—	M	M
YP9	—	—	M	M
YP13	(256–280)	(250–271)	(250–280)	(268–280)
YP16	M	M	M	—
YP17	(224–234)	(221–227)	(221–245)	M
YP28	—	—	M	M
YP30	—	—	(186–205)	(186–205)
YP41	(196–208)	(196–211)	(200–208)	—
YP49	—	—	(144–162)	—
YP55	—	(260–274)	M	—
YP60	(189–201)	(195–205)	(183–221)	M
YP62	—	—	M	(208–212)
YP65	(269–288)	(277–283)	—	—
YP66	—	—	(276–284)	—
YP68	—	(226–232)	(222–240)	—
YP71	—	—	M	—
YP73	—	(138–160)	M	(140–146)
YP78	(196–220)	(176–184)	(184–220)	—
YP79	—	—	M	(138–164)
YP80	M	M	M	—
YP81	—	—	—	M
YP84	—	—	M	M
YP85	(164–194)	—	—	—
YP96	—	(138–164)	—	—
YP99	(274–304)	—	—	—
YP106	(346–384)	(368–389)	—	(362–397)
YP108	(178–214)	—	M	(196–205)
YP110	—	(168–180)	(172–188)	—
YP111	(245–257)	—	(236–254)	—
YP113	(143–145)	M	(129–147)	—

The results of cross-species amplification (Table 2) revealed that the most transferable loci (25 of 32 loci) were amplified in *Perca fluviatilis*. Of the 32 loci developed, 60% amplified in the remaining three species (*S. canadense*, *S. vitreus*, *P. peltata*; 15, 16, and 14, respectively) with varying levels of polymorphism. As has been demonstrated for perch as well as a number of other fishes (O'Reilly & Wright 1995; Waters *et al.* 2000), these additional loci

will enhance ecological and population genetic studies, promote estimating phyletic relationships among perches, and facilitate parentage and pedigree analysis. These markers are being utilized currently in the Ohio Genetic Improvement of Farmed-fish Traits (O'GIFT) program for a yellow perch marker-assisted breeding scheme known as walk-back selection (Doyle & Herbinger 1994). Genetic map construction and inbreeding assessment are also possible areas of application for these markers.

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